PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 14544-PCT	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/IB2004/001049	International filing date *(day/month/year)* 05.04.2004	Priority date *(day/month/year)* 04.04.2003
International Patent Classification (IPC) or national classification and IPC C07K19/00, C12N15/62		
Applicant UNIVERSITE DE LAUSANNE et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 12 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 6 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04.02.2005	28.04.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Valcarcel, R Telephone No. +49 89 2399-2368

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IB2004/001049

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-47 as originally filed

Sequence listings part of the description, Pages

1-3 as originally filed

Claims, Numbers

1-41 received on 08.02.2005 with letter of 08.02.2005

Drawings, Sheets

1/19-19/19 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IB2004/001049

Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   - ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☒ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-40 (all partially); 21-27 (with regard to industrial applicability)

   because:

   ☒ the said international application, or the said claims Nos. 21-27 (with regard to industrial applicability) relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-40 (all partially)

   ☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

   ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

   ☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☐ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☐ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☐ neither restricted nor paid additional fees.
2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is
   - ☐ complied with.
   - ☒ not complied with for the following reasons:

     see separate sheet
4. Consequently, this report has been established in respect of the following parts of the international application:
   - ☐ all parts.
   - ☒ the parts relating to claims Nos. 1-40 (all partially), 41 .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-41
	No:	Claims	NONE
Inventive step (IS)	Yes:	Claims	NONE
	No:	Claims	1-41
Industrial applicability (IA)	Yes:	Claims	1-20,28-41
	No:	Claims	NONE

2. Citations and explanations (Rule 70.7):

   see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

The document numbering corresponds to the order of citation in the search report.

Re Item III

1. According to Rule 66.1(e) PCT, claims relating to inventions in respect of which no International Search Report (ISR) has been established need not be the subject of international preliminary examination. Accordingly, **no preliminary examination is carried out for these claims relating to the non searched inventions (inventions 2 to 10** as identified in the ISR, corresponding to amended claims 1-40, all partially).

2. Claims 21-27 are directed to methods of treatment of the human/animal body. Claim 31 is directed to a diagnostic method which could be practised on the human/animal body. Thus, claims 21-27 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item IV. Lack of unity

1. Eleven different (groups of) potential inventions had been recognised (see list in the ISR). The Applicant elected to pay an additional search fee without protest corresponding to the subject-matter of potential invention 11 (claim 45). Thus, inventions 1 and 11 were searched.

2. The subject-matter of inventions 1 and 11 lacks unity, contravening the requirements of Rule 13 PCT. Rule 13.1 PCT states that for unity of invention to be present, all subject-matter should be linked by a single general inventive concept. Rule 13.2 PCT stipulates that where a group of inventions is claimed the requirement of unity shall be fulfilled only where there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. **"Special" technical features** are those features that define a contribution which each of the inventions makes over the prior art.

   The common concept (technical relationship) linking **groups 1 and 11** together, with due consideration paid to the content of the description section, is the concept of enhancer sequences. It is noted that in claim 1, "enhancer sequences" in general are

referred to, and not the enhancer sequences of claim 41.

3. Enhancer peptide sequences were known in the prior art (see abstract of D11 and D12). In D11 the enhancer peptide improves the pharmacokinetic properties of the fusion protein. In D12 the enhancer peptide codes for a transport peptide which is responsible for an enhancement in the production of the protein. Both enhancers are understood to fall under the definition of enhancer in claim 1 of the present application.

4. Therefore, there is lack of unity among the two searched inventions (1 and 11). However, the IPEA has elected to carry out preliminary examination for both potential inventions without inviting to pay an additional examination fee.

Re item V

Invention 1

1. Claim 1 is unclear (contravening the requirements of Article 6 PCT). Claim refers to an **"enhancer sequence"** but no definition is given as to which kind of "enhancer" is referred to. For the purposes of preliminary examination, any enhancer sequence has been considered (see e.g. the abstracts of D11 and D12).

   Also the expression "humanized" in section (a) of claim 1 is unclear. The human protein has been considered also as "humanized". In view of claim 3, the Applicant also considers that the human form is also "humanized".

2. The present application does not meet the criteria of Article 33(1) PCT, because **the subject-matter of claims 1-40 does not involve an inventive step** in the sense of Article 33(3) PCT.

2.1 **D2 (considered as the closest prior art)** discloses isolated and recombinant fusion peptabodies expressed in E. coli, comprising human epidermal growth factor (hEGF). hEGF is known to bind ErbB-1 and ErbB-3 (see abstract of D4). **It is noted that hEGFR and ErbB-1 are synonyms** (see page 10, lines 28 and 29 of the present application).

The peptabodies of D2 comprise:
(a) a portion of cartilage oligomer matrix polypeptide (COMP) in particular the coiled-coil

region. In D2 it is not specified whether it was the human cartilage oligomer matrix polypeptide, but since it was used for human prostatic cancer cells, it is assumed that the human COMP was used;
(c) a portion of a hinge (a semirigid IG hinge region) at the C-terminal of the COMP;
(d) an epidermal growth factor receptor ligand at the C terminus of the hinge region: either **hEGF which binds hEGFR, and therefore ErbB-1** ; or a biogenic insect peptide of 25 amino acids named growth blocking peptide, GBP, which possesses the ability to interact with EGFR. It is noted that hEGF is very well known to have at least a motif having a three dimensional structure (see e.g. D3, D4, or paragraph bridging pages 8 and 19 of the present application).
The fusion peptabody of D2 is capable of inducing cellular death of cancer cells (see end of the results section).

2.2 **D1** discloses 3 isolated and recombinant fusion peptabodies (see figure 2). The peptabodies were expressed in E. coli and the recombinant proteins were purified (see D1, page 752, left column, third paragraph). The peptabodies of D1 comprise:

(a) a portion of the human cartilage oligomer matrix polypeptide (including 48 amino acid residues: see page 749, right column, second paragraph; figure 2; and page 752, left column, third paragraph), said portion is considered as a **"humanized"** cartilage oligomer matrix peptide;
(c) a portion of a hinge (a 17 amino acid peptide: see page 749, right column, second paragraph; figure 2; and page 752, left column, third paragraph) located at the C terminus of the portion of the cartilage oligomer matric polypeptide;
(d) an epidermal growth factor receptor ligand (MARSGL, MARAKE, or MSRTMS) comprising at least a motif having a three-dimensional structure (see page 752, left column, third paragraph; figure 2; and Table 1), and located at the C terminus of the hinge region. The three peptides included in the peptabodies having the sequences MARSGL, MARAKE, and MSRTMS (see figure 2 of D1) comprise the motifs MARSXX, MARXXX, and MSRXXX respectively (see figure 1 of D1). It is here noted that a hexapeptide has a three-dimensional structure. The fusion peptabody of D1 is capable of inducing cellular death of cancer cells (see figure 6 and page 753, left column, first three paragraphs).

Since the peptabody of D1 binds to ErbB-2 and not to ErbB-1, ErbB-3 or ErbB-4, and does not appear to comprise an enhancer, D2 has been considered as closest prior art (since it has one additional technical feature in common with the subject-matter of claim 1, that the ligand binds to ErbB-1, ErbB-3 or ErbB-4). However, D1 is also relevant for inventive step (see below).

2.3 The difference with the peptabody of claim 1 of the present application and the one of D2 is the enhancer sequence (feature b of claim 1).

The technical problem can be considered as the provision of an alternative peptabody capable of inducing cell death. The solution is the one recited in claim 1 of the present application. Said solution is not considered inventive for the following reasons.

2.4 D11 discloses enhancer peptides which improve the pharmacokinetic properties of the fusion protein (see abstract). In D12 the enhancer peptide codes for a transport peptide which is responsible for an enhancement in the production of the protein (see abstract). The skilled person in view of the teachings of D2 and D11 (or D12) would have been motivated to add an enhancer sequence to the peptabodies of D1. **Thus, claim 1 is not inventive.**

2.5 The same reasoning applies to claims, 2-4 and 6. Claim 6 refers to the peptabody where EGF (or fragments or variants thereof) is the EGFR ligand. EGF was one of the EGFR explicitly disclosed in D2 (see section 2 above). Thus, **claims 2-4, and 6 are also not inventive.**

2.6 **Claim 5 is also not inventive**. It appears that not all the sequences recited in claim 5 enhance protein production for any possible EGFR ligand. From Figures 25 and 26 of the present application only certain enhancer sequences appear to be active for particular peptides. For example, figure 26 of the present application discloses that for GBP, only two of these enhancers (E2, YSFE, and E4, YSFEDL) were active. Very similar enhancer sequences, having one amino acid more or less are completely inactive.

On the contrary, in example 7 of the present application it is disclosed that the enhancer

sequences E0-E7 were fused to the N-terminal part of kallikrein 2 gene or human serpin a1-antichymotrypsin gene and the production of the two proteins was increased (although no experimental data is shown).

Thus, although the enhancer sequences recited might be active for certain proteins (example 7), not all are active for others (Figures 25 and 26). It is possible that whether the enhancer is active or not could depend on the particular amino acid sequence context of the particular peptabody construct.

If products falling under the scope of a claim do not solve the technical problem posed (to provide peptides which enhance protein production), the claim as a whole must be considered as not involving an inventive step.

2.7 The use of cytotoxin in fusion proteins to be used in methods of treatment was disclosed in D13. The use of fluorescent labels is of standard use in the art. Thus said features are merely among several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to solve the problem posed. Thus, **claims 9-16 are also not inventive** in view of the teaching of either D1 or D2 combined with the teaching of D13.

2.8 EGF, and EGFRs were known to be expressed in different types of cancer and EGF had been associated with apoptosis (see e.g. D5, page 2; D1; and D2). However, EGF was known to induce and counteract apoptosis depending on the cell type and signalling context (see D5, page 2, or abstract of D15).

The use of the peptabodies of the present application for particular cancers such as head, neck, bladder or melanoma has not been exemplified. Thus, if the subject-matter of claims 17-40 were not trivial for the skilled person, such matter would have to be considered as not sufficiently disclosed.

Furthermore, EGF was known to induce apoptosis in at least certain cancers such as breast or esophageal cancer (see abstract of D16, or see D2). The skilled person would have tried to used said factors in methods of cancer treatment. Since the polymerization of a given molecule in peptabodies has been shown to be an effective way of enhancing

the biological activity of said factor (see, D1, D2, and any of D6 to D10) the skilled person would have tried to use said EGF peptabodies in methods of cancer treatment.

Even further, D1 discloses that the 3 anti-ErbB-2 peptabodies inhibited proliferation (or cell growth) of SK-BR-3 cells (see page 753, left column, first three paragraphs, and figure 6). The SK-BR-3 cells are originated from a breast carcinoma cell line from the ATCC (see D1, page 750, right column, second paragraph). SK-BR-3 cells express ErbB-2 (a epidermal growth factor receptor) as disclosed in D1, see page 754, right column, second paragraph). Thus, the peptabodies of D1 were also used to treat cancer cells.

The skilled person would have tried to optimize expression conditions and to use adequate carriers. Thus, **claims 17-40 are also not inventive.**

3. For the assessment of the present **claims 21-27 on the question whether they are industrially applicable**, no unified criteria exist in the PCT Contracting States. The EPO does not recognize as industrially applicable methods of treatment of the human body by surgery or therapy and diagnostic methods practised on the human or animal body. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Invention 11

4. Claim 41 is unclear contravening the requirements of Article 6 PCT, since it refers to enhancer sequences defined by their activity and sequence, and also to **"variants thereof"**. The expression "variants" is vague, rendering the scope of claim 41 unclear. For the purposes of preliminary examination only the particular peptide sequences have ben considered.

5. The present application does not meet the criteria of Article 33(1) PCT, because **the subject-matter of claim 41 does not involve an inventive step in the sense of**

Article 33(3) PCT. The particular peptides mentioned in claim 45 have not been found in the prior art to be disclosed as an enhancer. However **claim 41 is considered not to involve an inventive step** for the following reasons.

It appears that not all the sequences recited in claim 41 enhance protein production for any possible EGFR ligand. Figures 25 and 26 of the present application disclose that only certain enhancer sequences claimed in claim 41 are active for particular peptides. For example, figure 26 of the present application discloses that for GBP, only two of these enhancers (E2, YSFE, and E4, YSFEDL) were active. Very similar enhancer sequences, having one amino acid more or less are completely inactive.

On the contrary, in example 7 of the present application it is disclosed that the enhancer sequences E0-E7 were fused to the N-terminal part of kallikrein 2 gene or human serpin a1-antichymotrypsin gene and the production of the two proteins was increased (although no experimental data is shown).

Thus, although the enhancer sequences recited might be active for certain proteins (example 7), not all said enhancers are active for others (Figures 25 and 26). It is possible that whether the enhancer is active or not could depend on the particular amino acid sequence context of the particular peptabody construct.

If products falling under the scope of a claim do not solve the technical problem posed (to provide peptides which enhance protein production), the claim as a whole must be considered as not involving an inventive step.

1. An isolated and recombinant fusion peptabody, which binds to the epidermal growth factor receptor ErbB-1, ErbB-3 or ErbB-4, comprising:

(a) a portion of a humanized cartilage oligomer matrix polypeptide;
(b) an enhancer sequence located at the N terminus of the portion of the humanized cartilage oligomer matrix polypeptide;
(c) a portion of a hinge region of an immunoglobulin polypeptide located at the C terminus of the portion of the humanized cartilage oligomer matrix polypeptide;
(d) an epidermal growth factor receptor ligand located at the C terminus of the hinge region, comprising at least a motif having a three-dimensional structure, and whereby said isolated and recombinant fusion peptabody is capable of inducing cellular death in a cell expressing epidermal growth factor receptor.

2. The isolated and recombinant fusion peptabody of claim 1, wherein the member of the epidermal growth factor receptor is ErbB-1.

3. The isolated and recombinant fusion peptabody of claims 1-2, which is fully human or humanized.

4. The isolated and recombinant fusion peptabody of claims 1-3, wherein said isolated and recombinant fusion peptabody is multimeric.

5. The isolated and recombinant fusion peptabody of claims 1-4, wherein the enhancer sequence is selected from the group comprising: YSFE, YSFEDL, YSFEDLY, YSFEDLYR and YSFEDLYRR.

6. The isolated and recombinant fusion peptabody of claims 1-5, wherein said epidermal growth factor receptor ligand is selected among the group of:
(a) an epidermal growth factor polypeptide or fragments or variants thereof,
(b) a growth blocking peptide or fragments or variants thereof,
(c) a TGF alpha polypeptide or fragments or variants thereof,
(d) a plasmocyte spreading peptide or fragments or variants thereof,

(e) a paralytic peptide or fragments or variants thereof,

(f) a cardioactive peptide or fragments or variants thereof,

(g) an amphiregulin polypeptide or fragments or variants thereof,

(h) a heparin-binding epidermal growth factor-like polypeptide or fragments or variants thereof,

(i) a betacellulin polypeptide or fragments or variants thereof, or

(j) a viral EGF-like polypeptide or fragments or variants thereof.

7. The isolated and recombinant fusion peptabody of claim 6, wherein said epidermal growth factor receptor ligand is present in its full-length sequences.

8. The isolated and recombinant fusion peptabody of claims 1-7, further comprising a polyhistidine tag sequence.

9. The isolated and recombinant fusion peptabody of claims 1-8, further comprising at least one effector region.

10. The isolated and recombinant fusion peptabody of claim 9, wherein the effector region comprises a cytotoxin.

11. The isolated and recombinant fusion peptabody of claim 9, wherein the effector region comprises a detection moiety.

12. The isolated and recombinant fusion peptabody of claim 11, wherein said detection moiety is fluorescent.

13. An isolated and purified DNA sequence encoding the isolated and recombinant fusion peptabody of any one of claims 1-9.

14. A vector comprising at least one copy of the isolated and purified DNA sequence of claim 13.

15. The vector of claim 14, further comprising a promoter operably linked to said isolated and purified DNA molecule.

16. A prokaryotic or eukaryotic host cell capable of expressing the isolated and purified DNA molecule of claim 13.

17. A pharmaceutical composition comprising as an active substance a pharmaceutically effective amount of an isolated and recombinant fusion peptabody of claims 1-12 optionally in combination with pharmaceutically acceptable carriers, diluents and adjuvants.

18. Use of the pharmaceutical composition of claim17, for the preparation of a medicament for the treatment or prevention of cancer.

19. Use according to claim 18, wherein the cancer is selected from the group consisting of carcinoma, lymphoma, blastoma, sarcoma, liposarcoma, neuroendocrine tumor, mesothelioma, schwanoma, meningioma, adenocarcinoma, melanoma, leukemia, lymphoid malignancy, squamous cell cancer, epithelial squamous cell cancer, lung cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, testicular cancer, esophageal cancer, a tumor of the biliary tract, and head and neck cancer.

20. Use according to claim 19, wherein the cancer is head cancer, neck cancer, bladder cancer or melanoma.

21. A method of treating or preventing cancer that expresses epidermal growth factor receptors selected from the group consisting of carcinoma, lymphoma, blastoma, sarcoma, liposarcoma, neuroendocrine tumor, mesothelioma, schwanoma, meningioma, adenocarcinoma, melanoma, leukemia, lymphoid malignancy, squamous cell cancer, epithelial squamous cell cancer, lung cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer,

gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, testicular cancer, esophagael cancer, a tumor of the biliary tract, and head and neck cancer, comprising administering a therapeutically effective amount of the pharmaceutical composition of claim 17 to a subject.

22. The method of claim 21, wherein the cancer is head cancer, neck cancer, bladder cancer or melanoma.

23. A method for inducing apoptosis and/or necrosis, comprising contacting a cell with the isolated and recombinant fusion peptabody of claims 1-12.

24. The method of claim 23, wherein said cell is a cancer cell.

25. A method for inhibiting cell proliferation, comprising contacting a cell with the isolated and recombinant fusion peptabody of claims 1-12.

26. The method of claim 25, wherein said cell is a cancer cell.

27. A method of diagnosing cancer, comprising administering to a subject the isolated and recombinant fusion peptabody of claims 11-12, optionally in combination with pharmaceutically acceptable carriers, diluents and adjuvants.

28. A kit for treating cancer that expresses epidermal growth factor receptors in a human patient, said kit comprising the isolated and recombinant fusion peptabody of claims 1-12, optionally with reagents and/or instructions for use.

29. The kit of claim 28, further comprising a separate pharmaceutical dosage form comprising an additional anti-cancer agent selected from the group consisting of chemotherapeutic agents, anti-epidermal growth factor receptors antibodies, radioimmunotherapeutic agents, and combinations thereof.

30. A kit for diagnosing cancer that expresses epidermal growth factor receptors in a human patient, said kit comprising the isolated and recombinant fusion peptabody of claims 11-12, optionally with reagents and/or instructions for use.

31. A method for producing the isolated and recombinant fusion peptabody of claims 1-12, comprising the steps of:

a) constructing an isolated and purified DNA molecule encoding the isolated and recombinant fusion peptabody of any one of claims 1-12,

b) allowing expression of said isolated and purified DNA molecule in a cell system under suitable conditions,

c) recovering the isolated and recombinant fusion peptabody.

32. The method of claim 31, characterized in that the cell expression system is a prokaryotic cell.

33. The method of claims 31-32, characterized in that the suitable conditions consist in culturing the cell expression system at a temperature between 10-40 °C during 2-40 hours.

34. The method of claim 33, characterized in that the suitable conditions consist in a temperature of 37°C during 8-16 hours.

35. The method of claims 31-34, characterized in that step c) is achieved by extraction of said isolated and recombinant fusion peptabody from the cell expression system subsequently followed by purification and refolding steps.

36. The method of claim 35, characterized in that the purification is carried out in the presence of reducing agents and results in the elimination of contamination.

37. The method of claim 35, characterized in that the refolding step is carried out by direct dilution in refolding buffer and further comprises serial dialysis.

38. The method of claim 37, characterized in that the direct dilution in refolding buffer leads to a final concentration of the isolated and recombinant fusion peptabody below 300 nM.

39. The method of claim 37, characterized in that the serial dialysis comprise at least 2 different dialysis buffers.

40. The method of claim 37, characterized in that the refolding step consists in the oxidation of the isolated and recombinant fusion peptabody before its concentration.

41. A purified and isolated enhancer sequence having protein production increasing activity, characterized in that said purified and isolated enhancer sequence is selected from the group comprising: YSFE, YSFEDL, YSFEDLY, YSFEDLYR and YSFEDLYRR, a molecular chimera thereof and variants thereof.